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Review

Telomere length regulation in budding yeasts

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ABSTRACT

Telomeres are the nucleoprotein caps of chromosomes. Their length must be tightly regulated in order to maintain the stability of the genome. This is achieved by the intricate network of interactions between different proteins and protein–RNA complexes. Different organisms use various mechanisms for telomere length homeostasis. However, details of these mechanisms are not yet completely understood. In this review we have summarized our latest achievements in the understanding of telomere length regulation in budding yeasts.

1. Introduction

The ends of eukaryotic chromosomes, called telomeres, shield chromosomal DNA from the action of cellular nucleases. The next problem the telomeres solve is to prevent recognition as DSBs by the repair machinery, since this would lead to chromosomal fusions and eventually to loss of the genetic material. Both of these functions are achieved by the special organization of telomeres. Telomeric DNA, which consists of short GC-rich repeats, has a double-stranded region and a single-stranded G-rich 3′-overhang. These two regions are bound by specific sets of proteins, which distinguish telomeric chromatin from internal parts of the chromosome. Exact sequences of telomeric DNA and protein composition of telomeres vary between different organisms [1].

Telomeres shorten with each cell division due to what is known as the end-replication problem. RNA–protein complex telomerase reverse transcribes telomeric DNA, using its own RNA template, to counteract this problem [2]. Telomerase is crucial for the viability of unicellular eukaryotic organisms, such as ciliates and yeasts [3].

Genes adjacent to telomeres are subjected to silencing known as "telomere position effect" (TPE) [4]. Nevertheless, the subtelomeric regions contain promoters directed towards the ends of the chromosomes. The product of transcription from these promoters is a special class of non-coding RNAs called telomeric repeat-containing RNA, or TERRA [5]. TERRA is an important component of telomeric chromatin, as it participates in many aspects of telomere biogenesis [6]. However, its role is still poorly understood.

Budding yeasts are members of the subphylum *Saccharomycotina* of the phylum *Ascomycota* of the *Fungi* kingdom. Budding yeasts have proven to be useful models for studying diverse cellular processes. Research of telomere biology has been conducted on several representatives of the *Saccharomycotina* group. The relative evolutionary relationship between budding yeasts, that are described in this paper, is schematically depicted in Fig. 1.

Herein, we review mechanisms of telomere length regulation by telomerase and telomeric proteins in budding yeasts. The best studied organism in this field is *Saccharomyces cerevisiae*, so it will be the focus of our review. Comparisons with other model budding yeasts, such as *Candida albicans* or *Kluyveromyces lactis*, will be presented when possible.

2. Budding yeasts telomere structure

Telomeric repeats in budding yeasts have undergone dramatic changes during evolution: their sequences differ greatly from the canonical TTAGGG repeat (found in many organisms including mammals); their lengths lie within the 8–25 bp range, and telomeric repeats are often degenerate [7]. For example, *S. cerevisiae* repeats are heterogeneous $T(G)_{2\text{-}3}(TG)_{1\text{-}6}$, whereas *C. albicans* are homogenous ACGGATGTCTAACTTCTTGGTGT.

In *S. cerevisiae*, the double-stranded region of telomeres is bound by the Rap1 protein through its MYB domain [8]. Rap1

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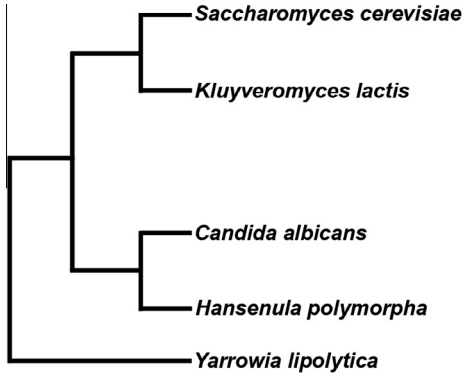

Fig. 1. The schematic representation of the phylogenetic relationship between budding yeasts described in the present work (adapted from [93]).

recruits two sets of additional proteins, interacting with its C-terminal domain. One of them (Sir3 and Sir4) is responsible for the TPE [9]. Another one (Rif1 and Rif2) is primarily required for telomere length regulation (Fig. 2A) [10,11]. Rap1 also prevents inappropriate exonuclease-mediated resection at telomeres and telomere fusions by inhibiting the non-homologous end-joining (NHEJ) repair pathway [12,13]. The single-stranded 3′-overhang is bound by Cdc13. Cdc13 together with its two interacting partners Stn1 and Ten1 forms trimeric RPA-like complex (CST complex) (Fig. 2A) [14]. The CST complex prevents recognition of telomeres by repair machinery and protects the C-strand from degradation by nucleases [15]. CST effectively competes with RPA for single-stranded telomere binding and inhibits RPA (and checkpoint kinase Mec1) accumulation. However, it does not affect binding of the MRX complex (and another checkpoint kinase Tel1) to DNA ends [16]. Apart from its capping function, Cdc13 regulates both G-strand and C-strand telomere synthesis, as it participates in both telomerase and replicative polymerase recruitment [17,18]. The Ku70/Ku80 heterodimer is another important telomere protein. Its exact location is still unknown, but it is thought to be the junction between double and single stranded regions of telomeric DNA (Fig. 2A) [19]. Ku70/Ku80 complex also plays role in preventing excessive C-strand resection [20,21]. *S. cerevisiae* has another MYB domain containing protein Tbf1 that binds TTAGGG sequences, located in subtelomeric regions (Fig. 2A) [22]. Tbf1 plays role in the regulation of the length of telomeres [23,24]. The MYB domain of Tbf1 is related to the MYB domains of mammalian telomere binding factors TRF1 and TRF2 [25].

Recent biochemical and structural characterization of Rap1, Rif1 and Rif2 binding to telomeric DNA suggests a model of a higher-order organization of telomeres. Through Rif1 tetramerization, polymerization of Rif2 and interaction of both with Rap1 all three proteins appear to be involved in the formation of a "Velcro"-like structure. Such organization provides necessary protection for telomeric DNA, but at the same time is dynamic, since it is composed of multiple weak interactions and can be easily disassembled [26].

Mammalian telomeres were shown to form a fold-back structure (t-loop) by interaction of the 3′-overhang with double-stranded telomeric DNA [27,28]. T-loop formation has been implicated in telomere capping. Although technical insufficiencies hamper visualization of t-loops in budding yeasts, and yeast telo-

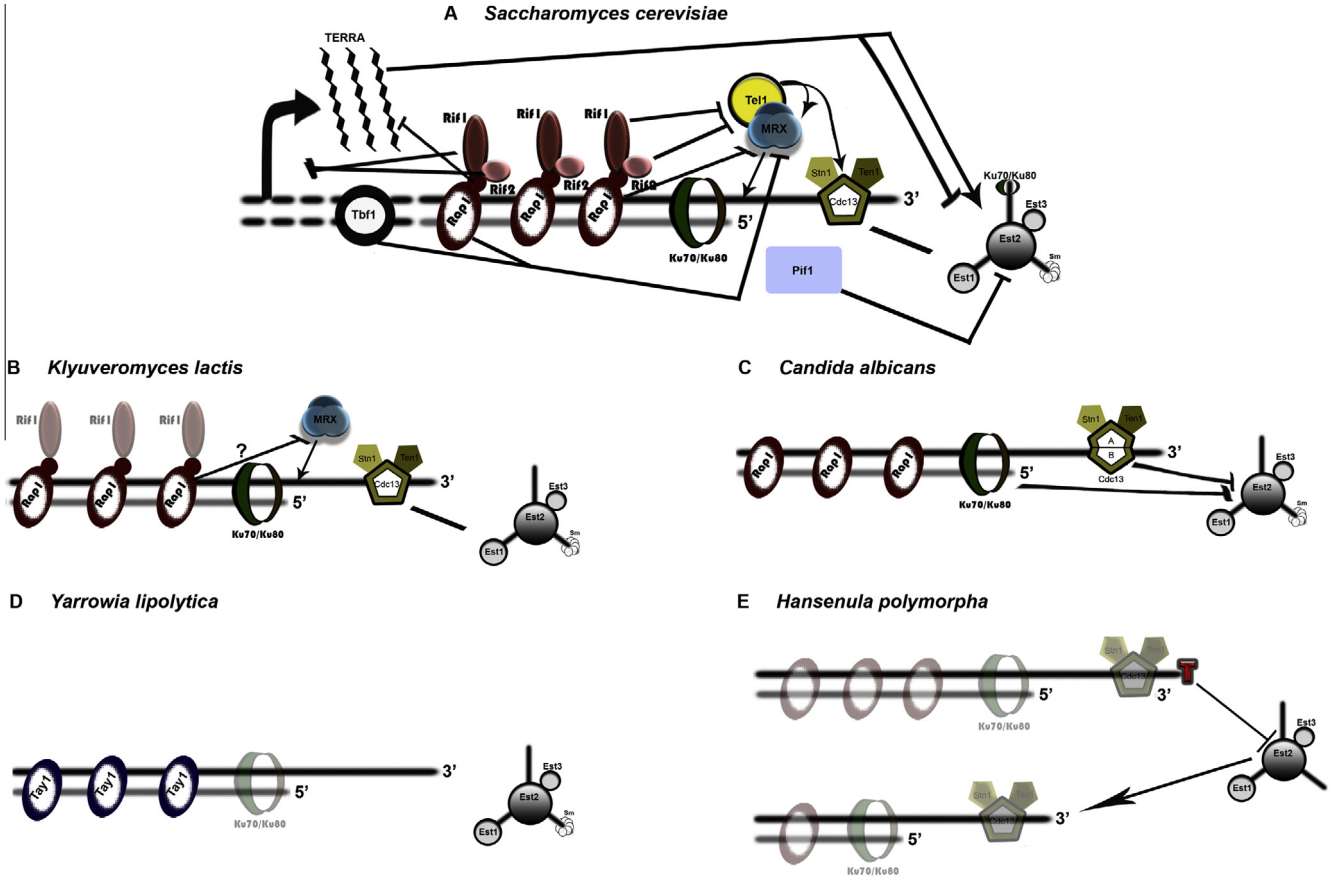

Fig. 2. Telomere structure and pathways regulating telomere-telomerase interaction in budding yeasts: (A) *S. cerevisiae*, (B) *K. lactis*, (C) *C. albicans*, (D) *Y. lipolytica*, (E) *H. polymorpha*. Black and grey parallel lines represent telomeric DNA, black dashed line represent subtelomeric DNA. Arrows represent activating effect, blunt arrows represent inhibitory effect. The line, connecting Cdc13 and Est1, represents their interaction. Lines, which connect Est2 and other components of telomerase, represent telomerase RNA. Proteins, the presence of which at telomeres is expected, but not confirmed, are lightened (in B, D and E).

meres are too short to allow 3′-overhang interaction with telomeric duplexes, much experimental evidence suggests that formation of a fold-back structure may occur at yeast telomeres (reviewed in [29]). In contrast to mammalian telomeres, yeast telomere loops involve subtelomeric regions.

In other budding yeast species, Rap1 is also thought to be the major double-strand telomere binding protein (Fig. 2). Rap1 homologues are present in many eukaryotic organisms, including fungi and mammals. However, in mammals and fission yeast, Rap1 does not bind DNA directly, and the DNA-binding activity of Rap1 from budding yeast is attributed to the duplication of a central MYB domain in these species [30]. Another two Rap1 domains (N-terminal BRCT domain and Rap1 C-terminal domain (RCT)) appear to be conserved. However, there are some exceptions. For example, *Yarrowia lipolytica* lacks an apparent Rap1 homologue (Fig. 2D) [31]. Interestingly, the RCT domain of Rap1 is absent in *C. albicans* (and several other *Candida* spp.), nevertheless CaRap1 binds double-stranded telomeric DNA in vitro with high affinity and specificity, and associates with telomeres in vivo (Fig. 2C) [32]. Since RCT plays a crucial role in mediating telomere length regulation in *S. cerevisiae* (through interaction with Rif proteins), its absence in some yeasts raises intriguing questions about Rap1 function in those species.

Y. lipolytica is the most distantly related to other studied budding yeasts. Its telomeres are bound by YlTay1 protein through its duplicated MYB domain (Fig. 2D). The MYB domain of YlTay1 is closely related to those of mammalian proteins TRF1 and TRF2 which contrasts with its poor similarity to other yeast MYB domain proteins, including ScTbf1. Curiously, *Y. lipolytica* appears to have a bona fide ScTbf1 homologue (named YlTbf1), but its possible telomeric role is uninvestigated yet. Experimental evidence of similarity between YlTay1 and mammalian TRF proteins are obtained from electron microscopy studies of YlTay1 interaction with *Y. lipolytica* telomeric repeats in vitro: for example in the formation of a structure resembling a mammalian t-loop [33,34].

Rif1 protein is conserved in other budding yeasts (also in other fungi, insects and vertebrates). Three conserved motifs were identified within Rif1: the HEAT repeat, SILK motif and a unique DNA binding domain, although the relative location of the domains varies between unicellular and multicellular eukaryotes. Direct DNA binding was demonstrated only for human Rif1 homologue, however yeast Rif1 also appears to contain a DNA binding domain, despite weak sequence similarity [35]. Rif2 and Sir3 are present exclusively in yeasts, which underwent whole genome duplication (*S. cerevisiae* and closely related species), and are thought to originate from Orc4 and Orc1 proteins [31].

Cdc13 (as well as Stn1 and Ten1) homologues have been identified in all budding yeasts with sequenced genomes, except for *Y. lipolytica*. *S. cerevisiae* Cdc13 has five domains [18,36]: the N-terminal OB1 serves for dimerization of ScCdc13 and Pol1 recruitment, RD (recruitment domain) mediates Est1–Cdc13 interaction, the presumable OB2 of unknown function, OB3 is a DNA-binding domain, OB4 is implicated in negative regulation of telomere length. Surprisingly, *C. albicans* (and several other *Candida* spp.) has two homologues of Cdc13 both shorter than ScCdc13, apparently lacking its two N-terminal OB-fold domains (including the recruitment domain). CaCdc13A and CaCdc13B are bona fide single-strand telomeric binding factors, as they bind the telomeric G-strand both in vitro and in vivo and their deficiency leads to defects in telomere regulation [37,38].

3. Telomerase in budding yeasts

The main components of the telomerase holoenzyme in all organisms are telomerase RNA and telomerase reverse transcriptase (in *S. cerevisiae* TLC1 and Est2, respectively). These constituents are sufficient for the activity of telomerase in vitro [39]. However, in vivo telomerase action requires a number of auxiliary factors, which differ greatly between organisms and it is often difficult to delineate their functional relevance to each other. In *S. cerevisiae* such factors are proteins Est1, Est3, Ku70/Ku80 and seven Sm proteins [36]. Est1 is crucial in telomerase recruitment to telomeres through its interaction with Cdc13 [40]. This protein also stimulates telomerase activity [41]. Est3 function is more elusive. It is thought to activate telomerase and modulate telomerase-DNA binding (perhaps in the anchor site), as it interacts with the TEN domain of Est2 [42,43]. Ku70/Ku80 is responsible for the nuclear import of TLC1, as it binds both telomeric DNA and telomerase RNA but in a mutually exclusive way [44]. The Sm complex protects the 3′-end of TLC1 [45].

Despite difficulties in discovering the genes of telomerase RNAs using ordinary bioinformatic approaches (due to high sequence divergence of TERs) the respective genes have been identified and characterized in many budding yeasts. The overall architecture of TERs from different yeasts seems to be conserved, as they have similar structural elements: a template region, template boundary element (TBE), pseudoknot and triple helix, three way junction and Est1-binding hairpin [46–48]. However, the presence of the stem-loop element, responsible for interaction with a Ku heterodimer, is confined in TLC1 and its most closely related species [49]. The main protein components of the telomerase complex (Est2, Est1 and Est3) are relatively conserved, and their homologues have been found to be encoded in genomes of all budding yeasts. The two exceptions are *C. parapsilosis* and *Lodderomyces elogisporus*, which do not have plausible Est1 homologues. In addition, Est3 telomerase subunits in these two organisms have N- and C-terminal extensions of unknown function [42].

4. Telomere length regulation in *S. cerevisiae*

CHIP measurements revealed that in *S. cerevisiae* Est2 and TLC1 are present at telomeres throughout the cell cycle with two peaks of binding: in G1 and late S phases [50]. Binding in G1 phase is dependent on TLC1–YKu80 interaction [51], whereas association with telomeres in S phase is due to Cdc13–Est1 interaction [52]. Telomerase acts on telomeres only in late S phase after the semi-conservative replication of telomeric DNA is complete [53]. This can be explained by the existence of different states of telomeres: in late S phase telomeres adopt an "open" state accessible for telomerase, but in other phases it is "closed". Est1 and Est3 proteins that are essential for telomerase action in vivo are detected at telomeres only in late S phase [50,54]. Rif proteins somehow regulate telomere accessibility for telomerase, in the *rif1* and *rif2* background telomeres are elongated in both G1 and S phases [55]. This is not surprising, given their involvement in the formation of a higher-order structure, as it has been suggested recently [26].

Several lines of evidence suggest that not all telomeres are processed by telomerase during every cell division, with short telomeres being better substrates for telomerase than longer ones. First, the rate at which telomeres are elongated is dependent on their length [56]. Second, the chance of being elongated in any given cell cycle is higher for short telomeres [57]. CHIP experiments also show greater levels of telomere bound Est1 and Est2 at short telomeres in late S phase [58,59]. Finally, live-cell imaging of TLC1 molecules allowed to propose that the telomere elongation event is localized and organized in clusters, containing few telomeres and several telomerase molecules [55]. Cells, which actively elongate telomeres, have the increased number of such clusters.

All these results fit with the protein-counting model which was proposed more than a decade ago [60] to explain the inhibitory

effect of the Rap1 protein (and its two interacting factors Rif1 and Rif2) on telomere length. More telomeric repeats would create more binding sites for the Rap1 protein, and Rap1 accumulation will drive long telomeres to a state that prevents telomere elongation, through the formation of a structure inhibiting telomerase binding or activity. Cdc13 was suggested to be the intermediate, transducing the signal to telomerase. Short telomeres would contain less Rap1 and hence more likely are in an extendible state.

The mechanism of inhibition of telomerase binding exerted by Rif proteins was found to be mediated mainly by Tel1 kinase (homologue of mammalian ATM kinase) (Fig. 2A) [61]. As in the case of DSB, Tel1 is recruited to telomeres through an interaction with the C-terminus of Xrs2 subunit of the MRX complex. Both MRX and Tel1 preferentially bind short telomeres [59,62,63]. Rif2 competes with Tel1 for the binding of the C-terminus of Xrs2, thus reducing Tel1 association with long telomeres. Rif1 also inhibits Tel1 binding, but through an unknown mechanism. Moreover, its inhibitory effect is smaller than that of Rif2, and is partially dependent on Rif2 [64]. It is worth noting that in *Δrif1* cells telomeres are longer than in *Δrif2* [10]. Thus, negative effects of Rif1 on telomere length cannot be explained by its effect on Tel1 accumulation. In *Δtel1* background MRX binding to DNA is reduced by Rap1 independently of Rif proteins (Fig. 2A). It is proposed that Rap1 would remove MRX complex from telomeres, after removal of Tel1 by Rif1 and Rif2 [64].

Tel1 kinase is thought to modulate telomerase recruitment at the level of Cdc13–Est1 interaction, with Cdc13 being the prime suspect for the target of Tel1 activity (Fig. 2A) [65]. However, only contradictory data exists on whether Cdc13 is phosphorylated by Tel1 or not [36,66]. An alternative model for the Tel1 function was proposed by Gao et al. [67]: absence of Tel1 results in a defective resection of the telomeric C-strand, which results in a suboptimal product for elongation by telomerase. This model can be expanded by the described above fact that Tel1 promotes MRX binding to telomeres, making it resistant to Rap1-dependent inhibitory action (Fig. 2A). In such a model, the focus of inhibition of telomere proteins is directed towards Mre11, rather than Tel1. In support of this model, *Δtel1* cells (with short telomeres) were shown to contain less ssDNA at telomeres, whereas Tel1-hy909 mutant (with elongated telomeres) improved telomeric ssDNA accumulation [68]. MRX complex and Tel1 kinase act as a single pathway of telomere regulation, and telomeres in *Δmre11* and *Δtel1* cells are equally short [69]. However, *Δmre11* cells contain more ssDNA than *Δtel1*, thus stimulation of MRX-dependent telomeric ssDNA generation may not fully explain Tel1 function in telomere length maintenance [69].

Another fact needs to be noted in the described mechanism of the protein-counting model: short telomeres contain as much Rif1 as long ones [59]. Together with the weak and Rif2-dependent inhibitory effect on Tel1, this leaves Rif1 function at telomeres totally enigmatic. Rif1 was found to inhibit accumulation of RPA (and subsequent checkpoint response) at uncapped telomeres, most likely through competition with RPA for the ssDNA [70]. Intriguingly, Rif1 association with DNA was not Rap1-dependent in this study. It would be interesting to test if the putative DNA binding domain of Rif1 is involved. It was proposed that Rif1 could similarly "hide" telomeric overhangs from telomerase, what would explain the inhibitory function of Rif1. Moreover, RPA itself has been implicated in telomerase recruitment through its interaction with Ku70/Ku80 and Est1 [71].

However, Rap1–Rif1–Rif2-dependent MRX/Tel1 inhibition is not the whole story with regard to telomere length control in *S. cerevisiae*. Transcription factors Tbf1 and Reb1 shorten telomeres, if their binding sites are placed near $TG_{1\text{-}3}$ repeats [23]. Most importantly, this effect is not attenuated (but rather is more pronounced) in *Δtel1* cells. Consistent with this, in the *Δtel1* background the frequency of elongation of a natural telomere, containing subtelomeric binding sites for Tbf1, was still length-dependent [72]. This effect can be explained by the fact that Tbf1 and Rap1 cooperate to inhibit accumulation of the MRX complex at DNA ends (Fig. 2A) [73]. Neither the RCT domain of Rap1, nor Rif1 and Rif2, are required for such inhibition, but rather an N-terminal region of Tbf1. The N-terminus of Tbf1 has been implicated in regulation of telomere length in other studies [23,72].

Telomeric $TG_{1\text{-}3}$ repeats can be substituted by the human-like TTAGGG repeats in strains bearing corresponding mutation in the TLC1 gene. Such "humanized" telomeres are bound by Tbf1 and are regulated in a length-dependent fashion, but independently of Rap1–Rif1–Rif2 and length-dependent regulation is unaffected by the presence or absence of Tel1 gene [73,74]. However, Tel1 kinase does play role in the maintenance of TTAGGG telomeres, since such telomeres are short in *Δtel1* cells [74]. In another study [75], MRX binding to DNA ends terminating in 230 bp of TTAGGG was shown to be inhibited by Tbf1, whereas short (60 bp) stretches of TTAGGG allowed higher MRX binding. Similarly, 60 bp TTAGGG telomeres bound more Est1 and Est2 compared to long telomeres. Telomerase binding at short TTAGGG ends was not affected by TEL1 deletion, however elongation of such ends was significantly reduced in a *Δtel1* background. This may explain why telomere length, but not length-dependent regulation, is affected in *Δtel1* yeast with "humanized" telomeres.

Another protein seems to contribute to the length-dependent inhibition of telomerase-telomere association – namely, Pif1 helicase [76]. It binds preferentially long telomeres and somehow reduces Est2 accumulation through interaction with the finger domain of Est2 (Fig. 2A) [36,77].

Abnormally elongated telomeres can be shortened to a wild type length through a telomere rapid deletion (TRD) event. This process occurs in wild type yeast at a relatively high rate: there is an ~4% chance that in any cell division one of the telomeres in a haploid cell may undergo rapid deletion [78]. The mechanism of TRD was shown to be the intrachromatid recombination: after 3′-overhang invasion into the centromere-proximal double-stranded telomere region the intervening sequences are excised [79]. Of note, the MRX complex is both a positive and a negative regulator of TRD [80].

The product of transcription of telomeric DNA (TERRA) provides another level of regulation of telomere length (Fig. 2A). In wild-type *S. cerevisiae* cells, TERRA levels are very low and its detection requires impairment of function of Rat1 exonuclease. *rat1-1* mutants, accumulating TERRA, have short telomeres, and this telomere shortening is due to telomerase inhibition [81]. In another study induction of TERRA transcription from a single telomere led to its shortening, however, this shortening was independent of telomerase activity. In this case TERRA was found to impede Ku70/Ku80-dependent protection of telomeres from degradation by Exo1 exonuclease [82]. These experiments indicate that TERRA is a negative regulator of telomere length.

Rap1 protein was found to regulate TERRA levels by multiple pathways [83]. The RCT domain of Rap1 stimulates Rat1-dependent degradation of TERRA. In addition, Rap1-interacting proteins inhibit TERRA transcription. Such inhibition is telomere specific, since TERRA levels from telomeres, containing only X elements in their subtelomeric region, is dependent mostly on Sir proteins, whereas TERRA repression at Y′-containing telomeres was primarily dependent on Rif proteins. Of note, Rif1 exerted a stronger inhibiting effect on TERRA than Rif2.

An unexpected result was obtained from experiments on live cell imaging of TERRA [84]. Such experiments revealed that about 10% of cells expressing GFP tagged TERRA from a single telomere, contain TERRA foci near nuclear periphery; and in S phase these foci colocalized with telomeres from which TERRA was transcribed.

Tagging of TLC1 molecules with MS2 repeats, telomere 6R with TetO repeats and the 6R telomere-derived TERRA with PP7 repeats allowed simultaneous visualization of these three molecules in vivo. It turned out that TERRA- and TLC1-containing foci were colocalized during S-phase prior to their colocalization with the 6R telomere. Furthermore, induction of TERRA transcription was shown to occur preferentially at short telomeres, which are good substrates for telomerase. These experiments allowed the authors to propose a model. According to the model, telomere shortening induces TERRA transcription, TERRA forms a focus, which in turn nucleates the formation of telomerase elongation-competent clusters, which are recruited subsequently to short telomeres. In this model TERRA acts as a (key) positive regulator of telomere length, which contradicts its previously characterized roles in the promotion of telomere shortening [85].

5. Telomere length regulation in other budding yeasts

Mutations in the portion of the template region of *K. lactis* TER, which encodes the KlRap1 site, cause uncontrolled telomerase-dependent lengthening of telomeres [86,87]. Upon introduction in telomeric DNA these mutations disrupt KlRap1 telomere binding, and overexpression of KlRap1 at least partially suppress the long telomere phenotype observed in those mutants. These observations imply that KlRap1 controls telomere length in *K. lactis* (Fig. 2B). Deletion of the C-terminal 31 aa of KlRap1 (KlRap1-ΔC) also leads to telomere overelongation [88], revealing that KlRap1 exerts a negative effect through its C-terminus like its homologue in *S. cerevisiae*. Deletion of two components of the MRX complex (KlMre11 and KlRad50) results in stable short telomeres [89], further emphasizing the similarity between the telomere length regulation mechanisms of *K. lactis* and *S. cerevisiae* (Fig. 2B). However, the extent of telomere lengthening in KlRap1-ΔC strain is modest in comparison with drastic telomere deregulation observed in ScRap1-ΔC mutant [88]. This fact may point out the difference in requirements for the RCT domain in telomere length control in these two species. Since the main function of RCT in *S. cerevisiae* is inhibition of ScTel1 binding (through Rif proteins), it is conceivable to assume that KlTel1 plays minor (if any) role in telomeres, and KlRap1 can inhibit the KlMRX complex, similar to ScRap1 effect on ScMRX in *Δtel1* cells.

The telomeric repeat unit in *K. lactis* is relatively long (25 bp) and seems to contain several functional regions [86]. Two identical 5-bp regions at the edges of the repeats are necessary for proper translocation of telomerase [90]. Mutations in a region encoded by nucleotides 4–9 in the KlTER template lead to high levels of subtelomeric recombination, but have little influence on telomere length. Mutations in the right half of the KlRap1 site lead to slightly short telomeres (in contrast to mutations in the left half) and may represent a distinct region with a positive effect on telomere length [86]. At least some mutations in the region adjacent to the left half of KlRap1 site initially cause telomere shortening, but after cultivation of the mutant strains telomeres become extremely long [86]. KlRap1 binding to such mutant telomeres is not impaired and overexpression of KlRap1 does not suppress the lengthening phenotype. Furthermore, one of the mutations and KlRap1-ΔC have a synergistic effect on telomeres [88]. Long telomeres in the mutants can be observed in a *Δrad52* background, thus telomere elongation is not due to recombination, and most likely mutant telomeres are maintained by telomerase [86]. Although the described phenotype can be explained by the subtle influence on Rap1–DNA interaction, it may also open a possibility for the existence of another Rap1-independent mechanism of telomere length control in *K. lactis*.

Deletion of Rap1 in *C. albicans* leads to overelongated and heterogeneous telomeres [32]. Thus, CaRap1 and ScRap1 appear to share a function in mediating telomere length control. However, there are several aspects suggesting the existence of major differences in the mechanisms of function in these two proteins. ScRap1 prevents inappropriate telomere elongation through inhibition of telomerase, limiting its access to telomeres; and the RCT domain of ScRap1 is a crucial element in this process. A shorter CaRap1 protein lacks RCT domain of ScRap1, implying that another mechanism is involved. Moreover, telomerase is not a target of CaRap1 inhibitory action, since CaTERT deletion does not suppress the phenotype of *rap1*-null mutant, but rather exacerbates it [32]. This suggests that CaRap1 inhibits excessive telomere recombination. Other telomere proteins in *C. albicans* appear to regulate telomeres in a similar manner, what can be deduced from shared phenotypes of *ku70*-, *stn1*-, *ten1*- and *rap1*-null mutants [32]. However, in *ten1* (and perhaps in *stn1*) mutant, longer than wild type telomeres are maintained by both telomerase and recombination [91]. In the *ku70* strain telomere, overelongation is entirely telomerase-dependent [92]. It is possible that in *C. albicans* accessibility of telomeres to telomerase is regulated by the CaKu70/Ku80 heterodimer and the CaCST complex, and the protein-counting (Rap1-counting) mechanism described for *S. cerevisiae* is not operating in *C. albicans* (Fig. 2C).

A fascinating mechanism of telomere length control in thermotolerant yeast *Hansenula polymorpha* was described recently [47]. *H. polymorpha* telomerase reverse transcribed one extra nucleotide past the predicted template region of HpTER. Such an event would block further elongation by telomerase, since the synthesized sequence could not be perfectly realigned to the beginning of the template. Consistently, the non-cognate nucleotide was observed exclusively at the very 3′ ends of chromosomes. HpTER mutants that allowed robust translocation had long and heterogeneous telomeres – a hallmark of disrupted length regulation. The presence of the non-cognate nucleotide at the ends of telomeres could be a mark for those which have been just processed by telomerase. Loss of terminal nucleotides as a result of incomplete replication or degradation will create a normal substrate for telomerase. Thus incorporation of the extra nucleotide could differentiate between shortened and normal-length telomeres, and regulate telomerase access; this could be an alternative for the formation of a proteinaceous structure (Fig. 2E). It is currently unknown which proteins (Rap1, Rif proteins or other) are involved in telomere maintenance in *H. polymorpha*. It would be interesting to investigate the relationship between the described mechanism and other known regulatory mechanisms in these species.

6. Concluding remarks

The complexity of telomere length homeostasis is still far from complete understanding even for such a well-studied organism as *S. cerevisiae*. In this review we have described several regulatory pathways known to control telomerase action at budding yeast telomeres; however, many mechanistic details are missing. For example, the undoubtedly central role of the MRX complex and Tel1 kinase in telomerase recruitment is still debated. Furthermore, the long non-coding RNA TERRA has been assigned to be a both positive and negative regulator of telomerase in *S. cerevisiae*. To link different regulatory elements is another challenge that is yet to be overcome.

In contrast to the large quantity of data available on *S. cerevisiae*, telomere biology in other model budding yeasts is only starting to develop. And some major differences can already be observed. Whereas *S. cerevisiae* predominantly employs the Rap1-counting mechanism, existence of auxiliary pathways suggests that these pathways may be central for telomere length regulation in other budding yeasts. *Y. lipolytica* are an exceptionally interesting species, since their telomere structure appears to differ drastically from that of other budding yeasts and perhaps is more "mammalian-like". Investigation of various model organisms is helpful,

since comparative analysis allows the revealing of general or specific features of mechanisms in molecular biology. Future studies in various budding yeasts will drive us to better understanding telomere and telomerase biology.

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